ELISA Analysis

OBJECTIVES

The ELISA method is suitable for determining diazinon and chlorpyrifos pesticides in different water matrices.

EQUIPMENT

- ELISA Microtiter Plate Reader
- ELISA Test Kits (SDI – chlorpyrifos and diazinon)
- Continuous Rotator
- Micropipette and Repeater Pipette or 5-mL Volumetric Pipette
- Distilled Water
- Parafilm
- Protective Gear: Nitrile Gloves, Lab Coat, Lab Glasses

STANDARD PREPARATION

- Label 5 20-mL scintillation vials with the appropriate standard concentrations.
- Prepare standards using the following table. Mix each standard thoroughly by using a vortex machine.
- Use Micropipettes or Volumetric Pipettes for these procedures. Do not use a graduated 10-mL pipette.

Chlorpyrifos		**Diazinon**	
Concentration	Preparation	Concentration	Preparation
1	10 uL stock into 10 mL distilled	0.5	50 uL stock into 10 mL distilled
0.5	5 mL 1ug/L with 5 mL distilled	0.2	2 mL 0.5 ug/L with 3 mL distilled
0.3	3 mL 1ug/L with 7 mL distilled	0.1	1 mL 0.5 ug/L with 4 mL distilled
0.05	0.5 mL 1ug/L with 9.5 mL distilled	0.03	0.3 mL 0.5 ug/L with 4.7 mL distilled

TEST PREPARATION

- Bring samples, standards and reagents up to test temperature (25°C).
- Prepare data sheet with 2 replicates of each standard, control, field samples and QA samples.

Determine how many wells are needed for the test, and make sure that all wells in a row are used. Additional QA samples, dilutions or replicates can be used to fill blank wells.

TEST PROCEDURE

- Arrange standards and samples in the same order that they will be added to the wells. This ensures that the time between samples will not be variable.
- Add 100 μL of each standard and sample to test wells. Attempt to add samples to the wells in an evenly timed fashion (i.e. one sample every 8 seconds). Use a new pipette tip for each sample.
- Add 100 μL Enzyme Conjugate to each well in the same order as samples. Note time of last addition.

•Cover the wells with parafilm and incubate on the rotator for 60 minutes. Note time for rinse.

• At rinse time remove parafilm, vigorously shake contents into sink, and rinse 5 times with cool tap water. Shake to empty between rinses and tap wells against dry paper towels after final rinse.

• Add 100 μL Substrate to each well in the same order as samples. Cover with parafilm and place on rotator for 30 minutes. Note time of last addition and note time for Stop Solution addition.

• Add 100 μL stop solution to each well in the same order as the samples.

SPECTROPHOTOMETRIC MEASUREMENT

• Read the results of the ELISA tests within 30 minutes of the addition of Stop Solution.

• Turn on the plate reader and follow the instructive prompts. The reader should be set up correctly and no adjustments should be necessary. In adjustments are necessary, consult the user manual.

• Blank the reader on air, insert the carrier, and begin reading the samples using the primary wavelength.

• Select the second wavelength, blank on air, and begin reading the samples again.

• As the results appear in the readout, write them in on the data sheet.

QUALITY CONTROL

Several quality control parameters must be measured during each ELISA event:

• E Standard – A known standard that is prepared or obtained from a third party. We create the E Standards from stock solutions that are prepared from certified pure chlorpyrifos and diazinon. To prepare E Standard stocks: weigh out 25 mg Chlorpyrifos into a 100-mL volumetric flask. Pipette 25 μL Diazinon into a 100-mL volumetric flask. Fill with methanol and mix with a vortex machine. For both chemicals, pipette 10 μL of stock into a 100-mL volumetric flask. Fill with methanol and mix with a vortex machine. Aliquot into 4-mL vials and place in freezer for single uses. Prepare E Standards by mixing 100 μL secondary stock from vials into 10 mL distilled water. Final concentration is 0.25 ppb.

Frequency: Every analysis event.

• Duplicate Sample – This is an ELISA measurement of the duplicate toxicity sample.

Frequency: Every analysis event.

• Matrix Spike – This is a laboratory fortified environmental sample. Fortify one field sample using the E Standard procedure. This measurement can be compared to the original sample.

Frequency: Every analysis event.

• Bottle Control and Field Control – These can be combined into one treatment. Take a bottle of distilled water into the field and at a designated site transfer the contents to another bottle. Bring this sample back to the lab for analysis.

Frequency: Once per project.